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Effects of Divalent Metal Cholorides on Respiration and Extractable Enzymatic Activities of Douglas-Fir Needle Litter

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16. ABSTRACT

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BRIAN P. SPALDING

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Effects of Divalent Metal Chlorides on Respiration and Extractable Enzymatic Activities of Douglas-Fir Needle Litter[1]

BRIAN P. SPALDING[2]

ABSTRACT

The rates of CO_2 evolution from Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) needle litter, following application of divalent metal (Hg, Cd, Pb, Ni, Zn, and Cu) chlorides at rates of 10, 100, and 1,000 μg/g and Ca chloride at 7, 68, and 683 μg/g, were monitored at 2- to 3-day intervals for 4 weeks; extractable enzymatic activities were also measured on additional samples at 1 day, 2, and 4 weeks following treatment. All metals except Pb inhibited respiration when applied at the highest level. Cellulase activity was depressed by treatments of 1,000 μg/g of either Hg or Cd after 4 weeks but was not affected initially (1 day after). After 4 weeks, xylanase activity was also depressed by the 1,000-μg/g Hg treatment. Apparently these enzymatic activities declined due to decreased enzyme synthesis associated with inhibited microbial growth rather than by direct enzymatic inhibition. Although addition of Hg or Cu chlorides directly to litter extracts caused marked inhibition of most enzymatic activities, equivalent levels, when applied to the needle litter, produced no inhibitions in extracts after 1 day, except for invertase activity. Invertase activity was depressed at all intervals and β-glucosidase activity was elevated at all intervals by Hg at the 1,000-μg/g level. The level of extractable ninhydrin-reactive compounds was elevated by this Hg treatment at all intervals similar to the effect of fumigation of soil or litter. Regardless of treatment, cellulase and xylanase activities were correlated (r = 0.70, n = 192).

Additional Index Words: **heavy metals, litter decomposition, enzyme inhibition.**

The impact of heavy metals on forested areas leads to a retardation of litter decomposition (Ruhling and Tyler, 1973; Jordan and Lechevalier, 1975; Jackson and Watson, 1977; Bond et al., 1976). The reduced microbial activity, leading to the observed reduction in decomposition rate, can result from three distinct mechanisms. Firstly, heavy metals can be directly toxic to microorganisms (e.g., Sommers, 1960). Secondly, the metal complexes of litter components may be less susceptible to microbial decomposition (Martin et al., 1966). Thirdly, enzymatic activity, which releases assimilable substrate from plant litter, could be directly inhibited since metals, particularly Hg, Cd, and Ag, are general enzyme inhibitors. Tyler (1974) found that the activities of urease and phosphatase in Spruce mor were depressed in samples containing high levels (40 mg/g) of Cu and Zn near a Swedish brass foundry; these enzymatic depressions were likely due to decreased rates of enzyme synthesis associated with a depressed microbial population, rather than direct inhibition, since the Spruce forest had been impacted by the heavy metals for over 200 years. However, these effects are difficult to separate since enzyme inhibition ultimately leads to a depressed microbial population which results in a decreased rate of enzyme synthesis.

Recently, the activities of amylase, cellulase, and xylanase in extracts of coniferous leaf litter were found to be correlated with the rates of CO_2 evolution (Spalding, 1977). The present study was initiated to establish whether or not levels of heavy metals (Hg, Cd, Cu, Zn, Ni, and Pb), which were adequate to inhibit rates of CO_2 evolution from Douglas-fir litter, would also inhibit the extractable activities of enzymes, including amylase, cellulase, xylanase, invertase, β-glucosidase, and polyphenoloxidase. The effect of such metal treatments on total enzymatic activities of litter could well be quite different from any effect on extractable activities since the latter represents an undetermined fraction of the total. The effect of time following metal application was also studied to establish whether or not any inhibition occurred initially (implying direct enzyme inhibition) or after incubation of up to 4 weeks (implying a decreased rate of enzyme formation resulting from decreased microbial growth).

MATERIALS AND METHODS

Needle litter was collected on 20 Sept. 1976 from an approximately 20-year-old stand of Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco), in the McDonald Experimental Forest, Oregon State University School of Forestry, near Corvallis, Oregon. This litter was largely free from ground-layer vegetation and, due to the stand's young age, contained few woody plant parts. The litter was sorted by hand to remove the few twigs, cones, bark, soil aggregates, and stones prior to treatment and analyses. This needle litter contained 15% moisture and, when oven-dried (80°C, overnight), contained 22.8% ash (residue on ignition at 550°C for 2 hours). Analyses performed by Forest Sciences Laboratory, Pacific Northwest Forest and Range Experiment Station, Corvallis, Oregon, found this litter to contain 0.76% N, 0.12% P, 0.94% Ca, 0.38% Mg, and 0.27% K. Analyses performed by the Forage Analytical Service, Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon, showed this litter to contain 8.1 ppm Cu, 11.5 ppm Zn, 10.2 ppm Ni, 13.4 ppm Pb, and 1.3 ppm Cd. Analyses performed by the Corvallis Environmental Research Center, Laboratory Analytical Support Service, Corvallis, Oregon, indicated that this litter contained < 0.5 ppm Hg.

Weighed portions, equivalent to 2.50 g of oven-dry litter, were placed in 227-ml, polystyrene-foam tumblers and wetted with 6 ml of distilled water added dropwise to effect an even distribution. The tumblers were capped with molded-plastic lids, weighed (to monitor and correct moisture losses during the experiment), and incubated for 5 days before treatment with metal solutions.

Solutions of analytical reagent grade metal chlorides ($PbCl_2$, $CdCl_2$, $ZnCl_2$, $HgCl_2$, $NiCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, and $CaCl_2$) were prepared in water at a concentration of 0.250% metal. Dilutions of 1:10 and 1:100 of these solutions were also prepared. One ml of the initial solution, the 1:10 dilution, and the 1:100 dilution, when added to a litter sample, would yield an applied level of 1,000, 100, and 10 μg of metal per oven-dry g of litter, respectively. However, the $CaCl_2$ solution (0.171% Ca) and dilutions were prepared to yield applied levels of 683, 68, and 7 μg/g-Ca; on a molar basis, the 683 μg/g-Ca level is equivalent to the highest molar level of heavy metal used, i.e., Ni at

[1] Contribution from Corvallis Environ. Res. Center, Corvallis, OR 97330. Received 10 Apr. 1978.

[2] National Research Council Resident Research Associate, USEPA, Corvallis Environ. Res. Center, Corvallis. Present address: Environ. Sci. Div., Oak Ridge Natl. Lab. (Operated by Union Carbide Corp. under contract W-7405-eng-26 with the U.S. Dep. of Energy), Oak Ridge, TN 37830.

17.0 μmoles/g or 1,000 μg/g. To achieve a uniform distribution of the applied solution on the litter, it was applied as a mist by injecting 1 ml, in four portions, into the uptake tube of an atomizer and spraying the resulting mist onto the litter in the bottom of the tumbler with intermittant mixing of the litter with a stainless-steel spatula. After the final portion was applied, the uptake tube of the atomizer was washed out with two 0.25-ml volumes of water by spraying onto the litter. An initial experiment, applying a potassium permanganate solution to litter by this procedure, indicated that almost all colored spray was deposited on the litter, not the white-walled tumbler, and that all residual color was washed from the atomizer uptake tube with the two water washes. The treatment solution and washings raised the moisture content of the litter from 72% to its capacity, 76%.

Nine replicates were prepared of each of eighteen treatments (six metals × three levels). However, only three replicates were prepared of the three $CaCl_2$ treatments (levels). Three replicates of each treatment were monitored for CO_2 evolution by the alkali-adsorption method (Spalding, 1977) for 4 weeks employing 2- to 3-day intervals. Respiration was determined at ambient laboratory temperature (ca., 22 ± 2°C) and was expressed as μg of CO_2 evolved per oven-dry g per hour. Three replicates were analyzed at the end of 1 day and another three replicates were analyzed 2 weeks following treatment to assay cellulase, xylanase, amylase, and invertase activities and to measure the concentration of reducing substances (Spalding, 1977); β-glucosidase and polyphenoloxidase activities and the concentration of ninhydrin-reactive compounds were also measured on all samples (Spalding, 1978). The three replicates, whose respiration was monitored, were analyzed 4 weeks after treatment and analyzed as above. The three calcium level treatments were also monitored for respiration for 4 weeks, extracted, and analzyed as above. Triplicate control treatments (water only) were run with each group of treated samples. The experiment was performed in three sections (Hg-Cd-Zn-control, Cu-Ni-Pb-control, and Ca-control treatments) with each section spanning about 6 weeks.

Analysis of variance was performed with a one-way classification (treatment) at each time interval: 1 day, 2 weeks, and 4 weeks. Whenever an *F*-test indicated that a significant difference existed, means of treatments were compared using Duncan's Multiple Range Test. Linear correlation coefficients between all pairs of variables (except respiration) were computed for all samples.

To observe any possible direct inhibition of enzymatic activities, 1 ml of a metal chloride solution (50 μg/ml-metal) was added to 1 ml of litter extract (Spalding, 1977). This final concentration of 25 μg/g metal in the extract would equal the maximum possible concentration of metal in an extract of a 1,000-μg/g-metal treated litter. The assays for enzymatic activities and measurements of biochemical concentrations were performed as above. All analyses were performed in duplicate using the same stock litter extract. Controls, using 1 ml of water, were run concurrently.

RESULTS AND DISCUSSION

The effects of the applied metal chlorides on the rates of CO_2 evolution from Douglas-fir needle litter are summarized in Table 1. At an applied level of 100 μg/g, only Hg exhibited an inhibitory effect. However, all metals except Pb inhibited respiration at an applied level of 1,000 μg/g. Even Ca at 683 μg/g or 17.0 μmoles/g [μmolar levels of the other heavy metals were 17.0 (Ni), 15.7 (Cu), 15.3 (Zn), 8.9 (Cd), 5.0 (Hg), and 4.8 μmoles/g (Pb)] was adequate to inhibit respiration. In all cases, respiratory inhibitions could be observed in the first measurement interval (0-3 days) and continued at each interval throughout the 4-week incubation.

Activities of cellulase and amylase were not initially affected by any metal treatment (Table 2). Xylanase activity appeared to be stimulated initially by either Zn or Cd at 1,000 μg/g but this effect was not observed 2 or 4 weeks after treatment. Xylanase activity also appeared to be stimulated after 2 weeks by Pb at 100 or 1,000 μg/g or Cu at 1,000 μg/g but these treatments exhibited no effects initially or after 4 weeks. However, the extractable invertase activity, which was previously found to be unrelated to respiration rates of coniferous leaf litter (Spalding, 1977), initially was markedly decreased by Hg at both 100 and 1,000 μg/g (Table 3). This inhibition by Hg was sustained at 2 and 4 weeks in the 1,000-μg/g treatment. Notably, invertase was also the most strongly inhibited activity when Hg was added directly to litter extracts (Table 4). Invertase activity was also initially stimulated by 10 or 1,000 μg/g Zn and Cd but this effect disappeared at 2 and 4 weeks (Table 3).

After 2 weeks, cellulase activity decreased in samples treated with 1,000 μg/g of Hg; this lower cellulase activity was observed again at 4 weeks for 1,000 μg/g of Hg and, also, for 1,000 μg/g of Cd. Xylanase activity, which is strongly correlated with cellulase activity (Spalding, 1977, 1978), was also depressed after 4 weeks by Hg at the 1,000-μg/g level. Notably, it was Hg at this level which exerted the most marked effect on respiration rate (Table 1).

Although amylase activity exhibited the best correlation with respiration in the previous study of coniferous leaf litter (Spalding, 1977), it was affected only by Cd or Pb at 1,000 μg/g and, then, only 4 weeks after treatment (Table 2). Since neither Cd nor Pb directly inhibited amylase activity in litter extracts (Table 4), their effect on amylase activity after 4 weeks was likely through depressed enzyme synthesis. An analogous conclusion was reached by Cole (1977) who observed that amylase ac-

Table 1—Rate of CO_2 evaluation of Douglas fir needle litter following application of metal chlorides.

Treatment		Interval (days)			
Metal	Level	0-3	12-14	26-28	0-28
	ppm	μg/g per hour			
None	0	255	199	117	187
Zn	10	244	187	120	180
Zn	100	246	180	114	177
Zn	1,000	224*	168*	102*	162*
Hg	10	245	189	124	184
Hg	100	227*	179*	103*	167*
Hg	1,000	186*	117*	57*	117*
Cd	10	247	194	115	181
Cd	100	257	202	122	190
Cd	1,000	214*	141*	90*	143*
(Mean)		235	176	106	169
None	0	226	206	117	186
Ni	10	211†	189†	108†	171†
Ni	100	217	202	115	181
Ni	1,000	203*	178*	96*	160*
Cu	10	223	194	111	177
Cu	100	216	200	111	179
Cu	1,000	194*	171*	93*	150*
Pb	10	223	194	110	175
Pb	100	235	207	119	187
Pb	1,000	214	187	110	172
(Mean)		215	182	109	173
None	0	299	200	150	207
Ca	7	303	206	163	212
Ca	68	295	194	146	203
Ca	683	275*	179*	135*	187*
(Mean)		293	195	146	202

* Significantly different from the control treatment in the particular group of samples at the 5% level.

† One of the three samples of this treatment was deleted in computing the means due to extremely anomalous behavior.

Table 2—Activities of cellulase, xylanase, and amylase in extracts of Douglas fir needle litter following application of metal chlorides.

Treatment		Time (weeks)								
Metal	Level	1/7	2	4	1/7	2	4	1/7	2	4
	ppm	μmoles of reducing sugar released/g per hour								
		Cellulase†			Xylanase‡			Amylase§		
None	0	14.6	19.0	19.2	36.3	40.2	44.4	6.1	6.3	6.1
Zn	10	14.8	14.7	19.1	34.6	33.8	46.3	5.5	5.6	6.3
Zn	100	13.9	15.1	18.3	34.7	36.3	39.1	5.0	6.8	6.4
Zn	1,000	15.5	16.2	17.6	48.1*	44.3	51.3	5.8	6.1	5.9
Hg	10	16.7	14.7	22.6	34.2	34.8	50.8	6.9	5.9	6.1
Hg	100	14.5	17.6	22.4	36.4	45.4	50.3	5.3	7.1	5.6
Hg	1,000	15.2	12.1*	14.2*	43.2	38.5	32.7*	6.1	7.3	6.3
Cd	10	18.0	18.0	17.6	46.3	40.9	43.3	6.4	6.4	5.3
Cd	100	16.4	17.6	19.0	40.7	43.4	49.1	6.2	6.2	5.0
Cd	1,000	16.2	16.3	13.7*	49.5*	42.4	38.7	7.5	7.0	4.0*
(Mean)		15.6	16.1	18.4	40.4	40.0	44.9	6.1	6.5	5.7
None	0	16.5	14.8	13.1	44.1	37.1	37.2	6.5	6.8	4.8
Ni	10	16.9	15.3	13.9	49.0	40.8	31.8	7.4	7.2	4.6
Ni	100	15.4	14.6	15.2	46.0	44.4	35.0	6.4	5.9	3.3
Ni	1,000	15.6	16.3	16.5	41.9	47.5	42.7	6.2	6.7	4.3
Cu	10	15.9	15.0	12.1	48.0	38.0	29.7	6.7	6.8	4.8
Cu	100	17.2	14.2	15.7	48.3	42.9	46.1	7.1	5.6	3.4
Cu	1,000	16.4	17.7	13.0	44.3	52.7*	37.1	6.3	6.8	3.7
Pb	10	18.4	14.4	16.1	50.9	41.8	41.3	7.2	6.4	3.4
Pb	100	16.2	18.4	17.1	48.5	48.9*	42.9	6.2	7.0	3.3
Pb	1,000	15.7	16.0	13.8	39.9	49.8*	36.9	5.4	6.4	2.9*
(Mean)		16.4	15.7	14.7	46.1	44.4	38.5	6.5	6.6	3.8
None	0	n.d.¶	n.d.	15.3	n.d.	n.d.	25.9	n.d.	n.d.	4.6
Ca	7	n.d.	n.d.	14.5	n.d.	n.d.	30.8	n.d.	n.d.	5.7
Ca	68	n.d.	n.d.	16.8	n.d.	n.d.	29.8	n.d.	n.d.	5.6
Ca	683	n.d.	n.d.	16.0	n.d.	n.d.	32.1	n.d.	n.d.	5.1
(Mean)		n.d.	n.d.	15.7	n.d.	n.d.	29.7	n.d.	n.d.	5.3

* This value is significantly different from the control treatment at the 5% level.
† LSD (0.05) = 3.9.
‡ LSD (0.05) = 9.8.
§ LSD (0.05) = 1.4.
¶ n.d. = not determined.

Table 3—Activities of invertase, β-glucosidase, and polyphenoloxidase in extracts of Douglas fir needle litter following application of metal chlorides.

Treatment		Time (weeks)								
Metal	Level	1/7	2	4	1/7	2	4	1/7	2	4
	ppm	μmoles of reducing sugar released or catechol oxidized/g per hour								
		Invertase†			β-glucosidase‡			Polyphenoloxidase§		
None	0	7.9	6.5	5.9	4.0	3.6	2.9	1.2	1.2	0.8
Zn	10	9.2*	6.4	6.2	4.4	2.6	3.1	1.1	1.0	1.1
Zn	100	8.5	6.3	6.2	4.0	2.8	3.0	1.1	1.1	0.9
Zn	1,000	9.1*	6.7	5.9	4.2	2.5	3.4	1.3	1.1	0.9
Hg	10	6.6	6.6	5.9	4.0	2.5	3.3	1.2	1.2	1.2
Hg	100	1.6*	5.6	5.6	4.3	3.2	3.7*	1.1	1.3	1.3
Hg	1,000	2.4*	2.6*	2.1*	5.0*	5.3*	6.1*	1.4	1.2	0.7
Cd	10	9.4*	6.8	4.8	4.8	2.8	3.0	1.4	1.1	0.8
Cd	100	8.6	7.1	5.6	4.4	3.3	3.1	1.4	1.4	0.9
Cd	1,000	9.3*	6.9	5.8	5.1*	3.4	3.5	1.3	1.2	0.6
(Mean)		7.3	6.2	5.4	4.4	3.2	3.5	1.3	1.2	0.9
None	0	8.8	7.1	4.9	4.2	3.2	1.7	1.3	1.3	0.9
Ni	10	9.1	7.6	5.0	3.9	3.3	1.5	1.2	1.4	0.9
Ni	100	8.7	6.6	4.5	4.2	3.3	1.8	1.2	1.4	0.9
Ni	1,000	9.5	6.9	6.0	4.1	3.7	2.5	1.2	1.9*	1.2
Cu	10	8.8	7.6	5.0	3.7	2.6	1.5	1.2	1.1	0.8
Cu	100	9.1	6.6	5.3	4.3	3.1	2.1	1.5	1.5	1.1
Cu	1,000	8.3	7.4	4.9	3.8	4.0	2.0	1.1	1.6	1.1
Pb	10	8.8	7.2	5.3	4.2	3.3	1.9	1.3	1.3	0.9
Pb	100	8.3	7.8	5.2	3.7	3.6	2.0	1.1	1.8	0.9
Pb	1,000	8.5	6.7	5.0	3.7	3.8	2.1	1.0	1.6	1.0
(Mean)		8.8	7.2	5.1	4.0	3.4	1.9	1.2	1.5	1.0
None	0	n.d.¶	n.d.	4.3	n.d.	n.d.	1.5	n.d.	n.d.	0.6
Ca	7	n.d.	n.d.	4.7	n.d.	n.d.	1.6	n.d.	n.d.	0.6
Ca	68	n.d.	n.d.	4.9	n.d.	n.d.	1.8	n.d.	n.d.	0.6
Ca	683	n.d.	n.d.	4.5	n.d.	n.d.	1.6	n.d.	n.d.	0.6
(Mean)		n.d.	n.d.	4.6	n.d.	n.d.	1.6	n.d.	n.d.	0.6

* This value is significantly different from the control treatment at the 5% level.
† LSD (0.05) = 1.1.
‡ LSD (0.05) = 0.7.
§ LSD (0.05) = 0.4.
¶ n.d. = not determined.

Table 4—Inhibition of enzymatic activities and biochemical concentration determinations in Douglas fir needle litter extracts by metal chlorides at 25 μg/ml-metal.

Activity or concentration	Metal						
	Hg	Cd	Cu	Pb	Zn	Ni	Ca
	% of control activity or concentration						
Cellulase	85*	99	98	101	103	102	98
Xylanase	89*	97	89*	99	102	99	98
Amylase	50*	90	77*	94	94	99	99
Invertase	18*	39*	95	98	99	100	41*
β-glucosidase	83*	101	89*	101	103	101	100
Polyphenoloxidase	88*	96	81*	96	90	91	93
Ninhydrin-positive compounds	100	92	65*	99	98	92	89*
Reducing substances	94	99	107	101	103	107	101

* Significantly different from control at 5% level.

tivity synthesis in soil was several times more sensitive to Pb inhibition than was amylase activity itself.

β-glucosidase activity was elevated at each time interval after treatment with 1,000 μg/g of Hg and after 4 weeks by the 100 μg/g Hg treatment (Table 3). Initially, 1,000 μg/g Cd stimulated β-glucosidase activity but, unlike Hg, this effect was not sustained at 2 and 4 weeks. However, Hg and Cu inhibited β-glucosidase activity when added directly to the litter extract (Table 4) at an equivalent concentration. Notably Tyler (1974) found that levels of Cu and Zn up to 40 mg/g of spruce mor had no effect on total β-glucosidase activity, although these same levels markedly inhibited respiration.

At a concentration of 25 μg/ml, added directly to the litter extract, no metal affected the observed concentrations of reducing substances (Table 4). The concentration of reducing substances in litter extracts was depressed after 4 weeks by 1,000 μg/g Zn and elevated by 100 μg/g Cd (Table 5). Initially, 1,000 μg/g Hg elevated the concentration of reducing substances but did not sustain this effect at 2 and 4 weeks. Although an interference with the determination of ninhydrin-reactive compounds by Cu and Ca added directly to extracts was observed (Table 4), neither metal affected this determination when applied to the litter (Table 5). Mercury at the 1,000-μg/g level markedly elevated the concentration of extractable ninhydrin-reactive compounds at each time interval. This elevation by the Hg treatment is similar to the effect produced by fumigation of soil (Ladd et al., 1976) or litter (Spalding, 1978) with chloropicrin. This apparent release of ninhydrin-reactive compounds is likely due to autolysis of microbial cells after biocidal treatment. Cd and Zn at 1,000 μg/g also elevated the concentration of extractable ninhydrin-reactive compounds initially but did not maintain this effect at 2 and 4 weeks.

Although concentrations of Hg or Cu of 25 μg/ml added directly to litter extracts were adequate to inhibit most of the enzymatic activities (Table 4), these same levels, when applied to the litter, yielded extracts whose enzymatic activities (except invertase) were not initially affected (Tables 2 and 3). This latter observation likely indicates that substantial amounts of the applied metals were adsorbed or complexed by the litter, which typically has a cation-exchange capacity of 50–80 meq/100 g (Youngberg, 1966) and, hence, the soluble concentrations necessary for the inhibitions observed in

Table 5—Concentration of extractable ninhydrin-reactive compounds and reducing substances in Douglas fir needle litter following application of metal chlorides.

Treatment		Time (weeks)					
Metal	Level	1/7	2	4	1/7	2	4
	ppm	μmoles/gram					
		Ninhydrin-positives†			Reducing substances‡		
None	0	4.1	5.5	5.0	25.6	24.9	15.5
Zn	10	4.5	5.0	4.9	27.1	20.9	14.2
Zn	100	4.6	5.2	4.3	26.7	23.0	12.5
Zn	1,000	5.1*	5.3	4.8	25.4	20.4	9.7*
Hg	10	4.9	4.6	5.4	28.4	22.4	20.9
Hg	100	5.1*	4.7	4.4	25.5	21.5	17.2
Hg	1,000	6.3*	8.5*	8.2*	32.7*	23.8	14.2
Cd	10	5.0	5.7	5.3	29.9	24.1	18.5
Cd	100	4.8	5.8	5.8	29.5	24.7	21.5*
Cd	1,000	5.3*	5.5	4.2	29.2	22.4	10.8
(Mean)		5.0	5.6	5.2	28.0	22.8	15.5
None	0	5.6	4.7	4.8	31.6	16.3	18.7
Ni	10	6.1	5.1	4.4	28.6	18.3	14.6
Ni	100	5.6	5.2	5.1	29.7	18.5	18.5
Ni	1,000	5.7	4.8	5.7	31.0	16.5	20.2
Cu	10	5.6	5.3	4.7	28.6	17.4	16.1
Cu	100	6.3	4.9	5.5	33.1	17.0	20.6
Cu	1,000	5.0	4.9	4.3	28.0	20.8	12.5
Pb	10	6.1	5.4	5.5	30.1	21.7	18.7
Pb	100	5.7	5.8*	5.9*	26.2	23.6*	21.5
Pb	1,000	5.6	5.5	5.4	29.0	22.4	18.1
(mean)		5.7	5.2	5.1	29.6	19.3	18.0
None	0	n.d.§	n.d.	5.1	n.d.	n.d.	16.1
Ca	10	n.d.	n.d.	6.2	n.d.	n.d.	17.6
Ca	100	n.d.	n.d.	6.1	n.d.	n.d.	16.3
Ca	1,000	n.d.	n.d.	6.1	n.d.	n.d.	13.5
(Mean)		n.d.	n.d.	5.9	n.d.	n.d.	15.9

* This value is significantly different from the control treatment at the 5% level.
† LSD (0.05) = 0.8.
‡ LSD (0.05) = 5.3.
§ n.d. = not determined.

Table 4, were not attained in litter extracts when the metals were added to the litter.

Since the respiration rate of the microbial population in needle litter declined immediately following the application of each metal except Pb, and since enzymatic activities, which were previously correlated with respiration, including amylase, cellulase, and xylanase, were not initially affected, a direct inhibition of these enzymatic activities cannot explain the respiratory decline. The subsequent fall of cellulase and xylanase activities 4 weeks after Hg or Cd treatment (Table 2) would suggest that these activities declined as a result of decreased enzyme synthesis associated with the inhibited microbial respiration. Both cellulase and xylanase activities in Douglas-fir needle litter have been observed to increase and subsequently decrease following inoculation of biocidally treated litter, indicating that the level of these activities is linked to microbial growth (Spalding, 1978). Although the mechanism of the metal-inhibition of microbial growth observed here cannot be completely established, some reasonable speculations can be advanced based on the present and related observations.

The complexing of metals with plant polysaccharides is known to decrease their susceptibility to microbial decomposition in soil (Martin et al., 1966). On the other hand, the complexing of metals with microbial cells themselves leads to decreased growth due to toxicity

Table 6—Linear correlation coefficients between biochemical variables of Douglas fir needle litter treated with metal chlorides.

Variable	Amylase	Xylanase	Cellulase	Invertase	β-glucosidase	Polyphenoloxidase	Reducing substances
Amylase	–						
Xylanase	0.41**	–					
Cellulase	0.40**	0.70**	–				
Invertase	0.41**	0.40**	0.22**	–			
β-glucosidase	0.56**	0.45**	0.21**	0.35**	–		
Polyphenoloxidase	0.52**	0.61**	0.40**	0.41**	0.45**	–	
Reducing substances	0.43**	0.35**	0.23**	0.54**	0.58**	0.49**	–
Ninhydrin-reactive compounds	0.30**	NS†	NS†	NS†	NS†	NS†	0.30**

** Significant at the 1% level; $N \geq 192$ in all cases.
† NS = not significant.

(Sommers, 1960). Both a starvation, i.e., decreased substrate decomposibility, and a toxicity mechanism would lead to decreased microbial respiration and enzyme synthesis (hence, decreased activity) observed in this study. Tyler (1975) found that both glucose and egg albumin were readily metabolized by both control and polluted (up to 40 mg/g of Cu and Zn) spruce mor samples, indicating that new microbial growth was not inhibited at the high metal concentrations; this ready metabolism at high metal concentrations could well be mediated by a metal-tolerant fraction of the microbial population. There is some evidence that zinc impaction of forest litter near a smelter led to selection of a more zinc-tolerant microbial population (Jordan and Lechevalier, 1975). But Tyler's (1975) observations are also consistent with the litter-metal complexing mechanism reducing substrate availability as well as a mechanism of direct microbial toxicity, although both mechanisms are likely to occur in metal-impacted forest litter.

The present study would also support such a starvation mechanism since even the Ca treatment of 17.0 μmole/g (683 μg/g) induced a respiratory decrease (Table 1). Since Ca is not toxic to fungi at such levels (Sommers, 1960), the operation of a complexing mechanism would seem to be a plausible explanation. An inhibition due to osmotic changes induced by such a Ca or other metal treatment cannot be ruled out since the addition of 683 μg/g of Ca as its chloride might exert an osmotic effect. A very informative experiment to either substantiate or discount the complexing mechanism would be an observation of decreased enzymatic hydrolysis of a metal-polysaccharide complex without concomitant loss in total activity toward the untreated polysaccharide. Such an observation would provide evidence for a microbial starvation mechanism since enzymatic activities, providing microbially assimilable substrate from plant litter, would be less effective when operating on metal-complexed litter.

Many of the enzymatic activities showed similar correlations with each other (Table 6) as were observed in the previous studies of a population of coniferous leaf litter samples (Spalding, 1977) or biocidally treated Douglas-fir litter (Spalding, 1978), if one considers that a considerably narrower range of activities was encountered in the present study. For example, the correlation coefficient between cellulase and xylanase activities, $r = 0.70$, is comparable to the previously observed $r = 0.91$, since a lower correlation coefficient would be expected when a similar scatter of data points about a regression line occurs within a narrower range. When the scatters of data points for paired observations were plotted, it was apparent that this was the case in the present study. Similar adjustments (about + 0.20) of the correlation coefficients in Table 6 for this narrower range of activities would elevate them to the level encountered in the previous study (Spalding, 1977).

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